THE SCIENTIST®

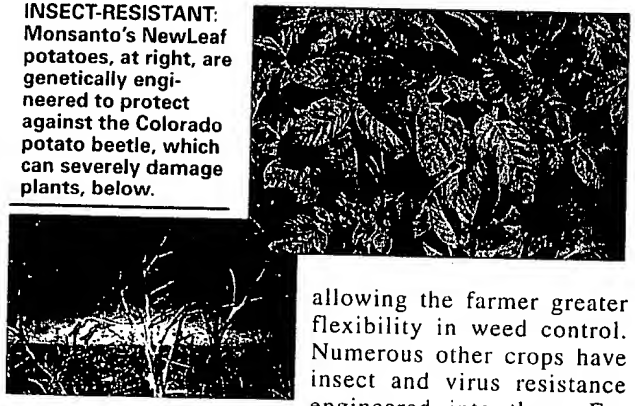
VOLUME 11, NO. 19 THE NEWSPAPER FOR THE LIFE SCIENCES PROFESSIONAL September 29, 1997

Political Controversy Puts Ag Biotech In Spotlight

BY JAMES KLING

Controversy is stirring in the European Union (EU) over genetically engineered food crops. The EU has already demanded that genetically engineered crops imported after July 31 be labeled as products of biotechnology. In the United States also, some consumers are wary of these new "supercrops," fearing that introduced genes could prove toxic or allergenic or that genes for herbicide resistance might encourage more chemically intensive agriculture. The labeling controversy in Europe threatens to interfere with U.S.-European trade, and a similar outcry in the U.S.—and resulting increases in government regulation—could strain research budgets and employment opportunities at agricultural biotech companies.

In answer to critics, companies and researchers point to the need for improved quality and increased quantity of food production to feed a skyrocketing world population. A popular example is the rice plant. The grain of this low-lying grass provides some 30 percent of the total calories consumed by the world's people. As productive as it is, the plant could be better—harsh climates, predatory insects, and invasive weeds all conspire to limit its yields. What's on U.S. and European markets: St. Louis-based Monsanto Life Science Co. offers Roundup Ready soybeans, which are resistant to Monsanto's Roundup herbicide,



INSECT-RESISTANT: Monsanto's NewLeaf potatoes, at right, are genetically engineered to protect against the Colorado potato beetle, which can severely damage plants, below.

allowing the farmer greater flexibility in weed control. Numerous other crops have insect and virus resistance engineered into them. For example, Monsanto's NewLeaf potatoes have been genetically engineered to include a strain of *Bacillus thuringiensis* (*Bt*)—a

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Embryonic Stem Cells Debut Amid Little Media Attention

BY RICKI LEWIS

Last July, with repercussions from Scottish sheep clone Dolly yet to die down, came news of potentially even greater importance. At the 13th International Congress of Developmental Biology in Snowbird, Utah, held the week of July 12, **John Gearhart**, a professor of gynecology and obstetrics at Johns Hopkins University School of Medicine in Baltimore, reported that he and postdoctoral fellow **Michael Shamblott** for seven months had cultured human embryonic stem (ES) cells. Such cells theoretically can divide and become virtually any cell type—bone or muscle, nerve or fibroblast, for example. That suggests intriguing applications, including a new tool to view development and even made-to-order human replacement parts.

STARTING POINT: Johns Hopkins' John Gearhart announced at a July meeting that he and a colleague had cultured human embryonic stem cells.

Developmental biologists, familiar with the central role of ES cells in making "knockout" mice, envisioned the ability to nurture human tissues in vitro on hearing of Gearhart's report. "I attended his talk, and I

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New Drugs, Devices Mount Assault On Diabetes

BY STEVE BUNK

Scientists are racing to find the grail of diabetes research, a therapy that will eliminate the need for insulin injection. New products, innovative clinical trials, and a boost in federal funding all are contributing to a multifaceted effort to control one of

ery systems," says **Frank Vinicor**, director of the division of diabetes at the Centers for Disease Control and Prevention in Atlanta. "Transplantation is another area."

Such R&D efforts are warranted: Diabetes is America's fourth leading cause of death by disease, costing an estimated $138 billion in direct health care and indirect productivity

value number of glucose levels for diagnosing diabetes. This measure, combined with wide-

ES Cells Debut

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was blown away by the possibilities," recalls **Leon Browder**, a professor of biology at the University of Calgary in Alberta, Canada. Adds **Thomas Doetschman**, a professor of molecular genetics at the University of Cincinnati College of Medicine who pioneered mouse ES cell technology (A. Gossler et al., *Proceedings of the National Academy of Sciences*, 83:9065-9, 1986): "The work is of considerable importance with respect to generating embryonic human material that could eventually be used to regenerate tissue in adults."

Yet the media took scant notice of the announcement. This puzzles Doetschman, who recalls the initial hoopla that greeted his earlier work. "When I first made ES cells in mice, people were very concerned about whether we could do this in humans. Then the interest died away. Perhaps now people are just tired from Dolly," he says. But **James Thomson**, an associate research veterinarian at the University of Wisconsin's Regional Primate Research Center in Madison, suggests another reason for the lack of coverage. "The press may be holding back because [the work] hasn't been published yet. When Gearhart demonstrates the three lineages [of specialized cells] developing from the cells, the press will pick up on it," he predicts. These are precisely the experiments that Gearhart is finishing now.

Two Routes To ES Cells

ES cells have been made from pigs, cows, rabbits, and sheep, and Thomson has cultured them from rhesus monkeys and marmosets (J.A. Thomson et al., *PNAS*, 92:7844-8, 1995; J.A. Thomson et al., *Biology of Reproduction*, 55:254-9, 1996). Human ES cells in long-term culture are something new.

Most nonhuman ES cells originate from the inner cell mass (icm), a collection of cells on the inside face of the blastocyst, the hollow-ball stage of early development. The icm forms layers and folds into the embryo. "To make ES cells, you are asking a few cells in the blastocyst to continue proliferating," says **Brigid Hogan**, a professor of cell biology at Vanderbilt University School of Medicine in Nashville and a Howard Hughes Medical Institute investigator. "As long as they keep on multiplying and not differentiating, you can go on growing them. This is what has been done in the rhesus monkey and marmoset."

LESSONS LEARNED: When Dyana Dalton of the Trudeau Institute first cultured ES cells from mice, she saw an embryoid body that looked like a heart.

But using blastocyst cells presents obstacles. They are difficult to manipulate. Plus, for work in humans, they must be "leftovers" from in vitro fertilization clinics, with informed consent from the donors. And researchers cannot use federal funds to perform experiments on embryos (K.Y. Kreeger, *The Scientist*, March 17, 1997, page 1).

Hogan and others devised an alternative route to obtaining ES cells, which Gearhart adopted, using private university funds. This approach uses embryonic germ, or EG, cells. "Once the embryo implants and starts developing, a few cells are set aside to give rise to the next generation of germ cells. They are protected from differentiating," explains Hogan. "If you culture cells from this region of the embryo, you can derive long-term cultures with all the properties of ES cells," adds Gearhart. He acquired EG cells from aborted five- to seven-week-old embryos, from a clinic.

But getting ES or EG cells is only the first step. Next, the cell culturist must reach into a bag of biochemical tricks to keep the cells from spontaneously forming embryonic structures. **Dyana Dalton**, an assistant member of the Trudeau Institute in Saranac Lake, N.Y., recalls the first time she cultured ES cells from mice for her work on interferon. "I was scanning small culture dishes, and out of the corner of my eye, I saw a heart-like object beating!" She had spied an embryoid body, which is a mass of differentiating tissue that forms from ES or EG cells. To block embryoid body formation, an investigator must add a cytokine called leukemia inhibitor factor (LIF) to the culture medium. "Otherwise, the culture will differentiate into most anything spontaneously. When you want differentiation, remove LIF and replace it with specific other cytokines, to regulate development," says Gearhart.

FUTURISTIC USES: Mouse ES cell technology could eventually be used to regenerate tissue in adults, says Cincinnati's Thomas Doetschman.

"Based on results with animal studies, it seems likely that we will be able to alter the cells so that a patient's immune system will not recognize them as transplants and reject them."

— John Gearhart, Johns Hopkins University School of Medicine

Skewing development toward musclehood or nervedom may not be as easy as mixing up a brew of biochemicals, according to Doetschman. "Under normal culture conditions, you might get 50 percent [of the cells yielding] embryoids, and 5 percent producing yolk sacs, which contain hematopoietic cells. You can tinker with the culture and maybe get 30 percent with a heartbeat [embryoids] and 50 percent hematopoietic. So far no one has ever been able to completely direct differentiation." The reason, he continues, is that an embryoid body is trying to recapitulate embryogenesis—not form a made-to-order liver for transplant purposes.

The human EG cells closely resemble true ES cells, Gearhart reports. He calls them ES-like cells. "The cells have a normal karyotype, and differentiate into several tissues. Now we are determining the various tissue types by doing transplants into immunocompromised mice," he states.

This technique was introduced to Thomson's work. The mice cannot reject the implants and provide a nurturing environment for cell specialization, says Thomson. "Working in nonhuman primates, I've seen muscle, cartilage, bone, teeth, and hair form," he adds.

Potential Applications

The ability to culture human ES cells presents tantalizing basic research opportunities. "Human ES cells would be very useful to understand the steps in differentiation. We could show, for example, which genes are turned on and which growth factors are necessary for cell specialization," explains Hogan.

ES cells stimulated to differentiate into human tissues or organs would provide a new type of model system to study disease and evaluate treatments. Although human genes have been introduced and expressed in mouse ES cells, assessing gene action in a human system would be a much better approximation of the human condition. "There are a lot of mouse models of human disease, but there is nothing like working with truly appropriate material," notes Gearhart. He developed human ES cells to assist his longtime research on trisomy 21 Down syndrome. "We would like to genetically engineer [ES] cells to increase the number of chromosome 21s, or fragments of it, and watch these cells form muscle or nerve," he adds.

MANY POTENTIAL USES: ES cells may someday be used as grafts to replace plaques in the brains of MS patients, predicts NINDS's Ronald McKay.

Ronald McKay, chief of the laboratory of molecular biology at the National Institute of Neurological Disorders and Stroke (NINDS), foresees use of ES cells as grafts to replace plaques in the brains of people with multiple sclerosis (MS). "You can take mouse ES cells and differentiate them in vitro into neuroepithelial cells, graft those into the brain, and get a large number of oligodendrocytes, glia, and neurons," he says. Researchers could theoretically add genes to tailor a graft to a particular individual or to ensure that healing cells migrate throughout the plaque. "Our work on mouse suggests that if we had bucketloads of human ES cells, we could indeed turn them into cells that we could implant into MS patients," McKay adds.

On the organ transplant front, ES cells could seed tissues that are either universal—accepted by anybody—or tailored to individuals. "Based on results with animal studies, it seems likely that we will be able to alter the cells so that a patient's immune system will not recognize them as transplants and reject them. If so, we would have a universal cell donor, cells that could be transplanted to any recipient," states Gearhart. Hogan explains how ES cells could be targeted to specific patients. "You might imagine gene manipulation to replace the MHC [major histocompatibility complex] of one cell with that of another, to create a bank of cell types, from kidney or bone marrow, for example." Eventually, she foresees, nuclear transplantation could be used to simply swap into an ES cell the nucleus from a person needing a transplant, then grow the tissue.

SEPARATE DECISIONS: Penn bioethicist Arthur Caplan says "the practice is morally defensible for most people if it only uses cells that would have existed anyway."

Perhaps the first transplant application will be bone marrow, because hematopoietic (blood-forming) tissue derives from yolk sacs, which are clearly seen in embryoid bodies. "One advantage of using human ES cells in bone marrow transplants might be that the more primitive cells are, the more plastic they are," notes **David Margolis**, an assistant professor at the Institute for Human Virology at the University of Maryland Biotechnology Institute in College Park. "In general, the more cell divisions and differentiation that cells have left, presumably the more they would be able to repopulate bone marrow." It would also take fewer ES cells to restore marrow than cells from current sources, which include umbilical cord blood and peripheral blood. Margolis genetically manipulates hematopoietic stem cells to treat HIV infection.

Bioethical Concerns

An attractive aspect of human ES cell-based technology, from an ethical standpoint, is that it could offer large-scale culturing of tissues and possibly organs, instead of conjuring up images of warehouses of bodies awaiting organ harvest. And unlike fetal tissue transplants used to treat Parkinson's disease, in which several fetuses are used for one adult patient, a few embryos could seed cultures of ES cells that could potentially help thousands of people. "The cells are a renewable resource. We could bank cells, without having to use additional abortion material," Gearhart maintains.

But the source of the starting material—"pregnancy terminations," or late-stage human embryos—may still disturb some people. **Arthur Caplan**, director of the Center for Bioethics at the University of Pennsylvania, counters such objections by separating the decision to abort from use of the tissue. "While some will object to the use of any fetal tissue simply on moral grounds with respect to elective abortion, the practice is morally defensible for most people, including me, if it only uses cells that would have existed anyway, and there is no connection between those doing abortions and those seeking starter cells for culture," he says.

Another ethical issue is whether human ES cells would be genetically altered and allowed to continue development into a newborn, as is done in mice. Anticipating such questions of germline manipulation and cloning, Gearhart says, "we will not perform any experiments aimed at genetically engineering the human germline in my lab or anywhere at Hopkins—it is not ethically acceptable. That's one of the reasons we chose to present our studies in an ethics forum [at Snowbird]. We wanted to begin the process of establishing a set of guidelines for the ethical use of cells of this type."

McKay considers use of ES cells as a new source of tissue grafts, comparable to blood transfusions and organ transplants. "Using ES cells is an obvious extension, but I don't think people should harvest embryos for grafts. I prefer the idea that we can expand cells with highly differentiated properties. In the abstract it may seem frightening, and the idea of harvesting embryonic tissue in an uncontrolled way may be distasteful. But if we could grow cells in the lab with certain useful properties, that would be a different, and very exciting, story."

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